

**Amendment and Response**

Page 2 of 17

Serial No.: 09/640,952

Confirmation No.: 3252

Filed: 17 August 2000

For: EPHA2 AS A DIAGNOSTIC TARGET FOR METASTATIC CANCER**Amendments to the Claims**

This listing of claims replaces all prior versions, and listings, of claims in the above-identified application:

**Listing of Claims**

1. (Previously presented) A method for detecting the presence of metastatic cells in a cell population comprising the steps of:
  - lysing at least a portion of the cell population,
  - incubating the lysed cells with a monoclonal antibody that specifically binds EphA2 to allow antibody binding to EphA2, and
  - detecting antibody-EphA2 binding;wherein the cell population comprises cancer cells selected from the group consisting of breast cancer cells, kidney cancer cells, prostate cancer cells, lung cancer cells, colon cancer cells and epithelial cancer cells; and wherein antibody-EphA2 binding is indicative of the presence of metastatic cells in the cell population.
2. (Canceled)
3. (Previously presented) The method claim 1 wherein the antibody binds to an intracellular epitope of EphA2.
4. (Previously presented) The method of claim 3 wherein the antibody is produced by hybridoma cell line D7 (ATCC number PTA 2755).
5. (Previously presented) The method of claim 1 wherein the antibody is labeled with a detectable label, and the detecting step includes detecting the label.

**Amendment and Response**

Page 3 of 17

Serial No.: 09/640,952

Confirmation No.: 3252

Filed: 17 August 2000

For: EPHA2 AS A DIAGNOSTIC TARGET FOR METASTATIC CANCER

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6. (Original) The method of claim 5 wherein the antibody is labeled with a fluorescent label and the detecting step comprises detecting the fluorescent label.
7. (Original) The method of claim 5 wherein the antibody is labeled with a radioactive label and the detecting step comprises detecting the radioactive label.
8. (Original) The method of claim 1 wherein the cell population comprises cells from a breast or prostate tissue biopsy.
9. (Original) The method of claim 1 wherein the cell population is harvested from a body fluid selected from the group consisting of blood, plasma, spinal fluid, saliva, and urine.
10. (Original) The method of claim 9 wherein the detecting step includes a diagnostic method selected from the group consisting of ELISA assays and flow cytometry.
11. (Original) The method of claim 1 wherein the incubating and detecting steps comprise western blotting methodology.
12. (Original) The method of claim 11 further comprising the steps of providing a second antibody having phosphotyrosine specificity, and western blotting with the second antibody.
13. (Original) The method of claim 1 wherein the metastatic cells are selected from the group consisting of breast, prostate, lung, and colon cancers.
- 14.-20. (Canceled)

**Amendment and Response**

Page 4 of 17

Serial No.: 09/640,952

Confirmation No.: 3252

Filed: 17 August 2000

For: EPHA2 AS A DIAGNOSTIC TARGET FOR METASTATIC CANCER

21. **(Currently amended)** A method for detecting the presence of metastatic cells in a tissue sample comprising the steps of

incubating the tissue sample with a reagent capable of specific binding to a nucleic acid coding for EphA2 protein, wherein said reagent comprises a nucleic acid that is complementary to the nucleic acid coding for EphA2 protein, to allow reagent binding to the nucleic acid, and detecting reagent-nucleic acid binding;

wherein the tissue sample comprises cancer cells selected from the group consisting of breast cancer cells, kidney cancer cells, prostate cancer cells, lung cancer cells, colon cancer cells and epithelial cancer cells; and wherein reagent-nucleic acid binding is indicative of the presence of metastatic cells in the tissue sample.

22. **(Canceled)**

23. **(Currently amended)** The method of claim 21 wherein the nucleic acid coding for EphA2 protein is DNA or RNA.

24. **(Previously presented)** The method of claim 21 further comprising, prior to the detecting step, the step of fixing the cells on a slide, wherein the detecting step comprises detecting the reagent-nucleic acid binding using immunofluorescence staining.

25.-32. **(Canceled)**

33. **(Previously presented)** The method of claim 5 wherein the antibody comprises at least one of a fluorescent label, a chemiluminescent label, a bioluminescent label, an enzymatic label, a chromogenic label and a radiolabel, wherein detecting antibody-EphA2 binding comprises detecting at least one detectable label.

34.-35. **(Canceled)**

## Amendment and Response

Page 5 of 17

Serial No.: 09/640,952

Confirmation No.: 3252

Filed: 17 August 2000

For: EPHA2 AS A DIAGNOSTIC TARGET FOR METASTATIC CANCER

---

36. (Previously presented) The method of claim 1 wherein the cell population comprises cells selected from the group consisting of breast cancer cells, kidney cancer cells, prostate cancer cells, lung cancer cells and colon cancer cells.
37. (Previously presented) The method of claim 1 wherein the cell population comprises epithelial cancer cells.
- 38.-40. (Canceled)
41. (Previously presented) The method of claim 1 wherein the cell population comprises cells from a tissue biopsy.
42. (Previously presented) The method of claim 41 wherein the tissue comprises breast tissue or prostate tissue.
43. (Previously presented) The method of claim 1 wherein the cell population comprises cells from a body fluid.
44. (Previously presented) The method of claim 43 wherein the body fluid is selected from the group consisting of blood, plasma, spinal fluid, saliva, and urine.
45. (Previously presented) The method of claim 1 wherein detecting antibody-EphA2 binding comprises utilizing a diagnostic method selected from the group consisting of an ELISA assay, a Western blot, and flow cytometry.
46. (Previously presented) The method of claim 1 wherein detecting antibody-EphA2 binding comprises utilizing a Western blot; the method further comprising Western blotting with a second antibody having phosphotyrosine specificity.

**Amendment and Response**

Page 6 of 17

Serial No.: 09/640,952

Confirmation No.: 3252

Filed: 17 August 2000

For: EPHA2 AS A DIAGNOSTIC TARGET FOR METASTATIC CANCER

---

47. (Previously presented) A method for detecting the presence of metastatic cells in a cell population comprising:

incubating at least a portion of the cell population with a monoclonal antibody that specifically binds EphA2 to allow binding of the antibody to EphA2; and

detecting antibody-EphA2 binding;

wherein the cell population comprises cancer cells selected from the group consisting of breast cancer cells, kidney cancer cells, prostate cancer cells, lung cancer cells, colon cancer cells and epithelial cancer cells; and wherein antibody-EphA2 binding is indicative of the presence of metastatic cells in the cell population.

48. (Canceled)

49. (Previously presented) The method of claim 47 wherein the antibody binds to an intracellular epitope of EphA2.

50. (Previously presented) The method of claim 47 wherein the antibody is produced by hybridoma cell line D7\_(ATCC number PTA 2755).

51. (Previously presented) The method of claim 47 wherein the antibody binds to an extracellular epitope of EphA2.

52. (Previously presented) The method of claim of claim 47 wherein antibody-EphA2 binding yields a bound complex comprising a whole cell.

53. (Previously presented) The method of claim 52 wherein detecting antibody-EphA2 binding comprises subjecting the bound complex to immunohistochemical staining.

**Amendment and Response**

Page 7 of 17

Serial No.: 09/640,952

Confirmation No.: 3252

Filed: 17 August 2000

For: EPHA2 AS A DIAGNOSTIC TARGET FOR METASTATIC CANCER

---

54. (Previously presented) The method of claim 47 wherein the antibody is produced by hybridoma cell line B2D6 (ATCC number PTA 2754).

55. (Previously presented) The method of claim 47 wherein the bound antibody comprises a detectable label; and wherein detecting antibody-EphA2 binding comprises detecting the label.

56. (Previously presented) The method of claim 47 wherein the bound antibody comprises at least one of a fluorescent label, a chemiluminescent label, a bioluminescent label, an enzymatic label, a chromogenic label and a radiolabel; and wherein detecting antibody-EphA2 binding comprises detecting at least one detectable label.

57.-58. (Canceled)

59. (Previously presented) The method of claim 47 wherein the cell population comprises cells selected from the group consisting of breast cancer cells, kidney cancer cells, prostate cancer cells, lung cancer cells and colon cancer cells.

60. (Previously presented) The method of claim 47 wherein the cell population comprises epithelial cancer cells.

61. (Previously presented) The method of claim 47 wherein the cell population comprises metastatic cancer cells.

62. (Previously presented) The method of claim 61 wherein the metastatic cells comprise cells selected from the group consisting of breast cancer cells, kidney cancer cells, prostate cancer cells, lung cancer cells, and colon cancer cells.

## Amendment and Response

Page 8 of 17

Serial No.: 09/640,952

Confirmation No.: 3252

Filed: 17 August 2000

For: EPHA2 AS A DIAGNOSTIC TARGET FOR METASTATIC CANCER

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63. (Previously presented) The method of claim 47 wherein the metastatic cells comprise epithelial cancer cells.
64. (Previously presented) The method of claim 47 wherein the cell population comprises cells from a tissue biopsy
65. (Previously presented) The method of claim 64 wherein the tissue comprises breast tissue or prostate tissue.
66. (Previously presented) The method of claim 47 wherein the cell population comprises cells from a body fluid.
67. (Previously presented) The method of claim 66 wherein the body fluid is selected from the group consisting of blood, plasma, spinal fluid, saliva, and urine.
68. (Previously presented) The method of claim 47 wherein detecting antibody-EphA2 binding comprises utilizing a diagnostic method selected from the group consisting of an ELISA assay, a Western blot, and flow cytometry.
- 69.-71. (Canceled)
72. (Previously presented) A method for detecting the presence of cancer cells in a selected cell population comprising:
- assaying at least a portion of the selected cell population for at least one of
    - a change in EphA2 intracellular localization pattern; and
    - a change in EphA2 phosphorylation content
  - as compared to the intracellular localization pattern and phosphorylation content in an analogous normal cell population;

**Amendment and Response**

Page 9 of 17

Serial No.: 09/640,952

Confirmation No.: 3252

Filed: 17 August 2000

For: EPHA2 AS A DIAGNOSTIC TARGET FOR METASTATIC CANCER

wherein the change is indicative of the presence of a cancer cell in the selected cell population.

73. (Previously presented) The method of claim 72 wherein a change in intracellular localization pattern or phosphorylation content is indicative of the presence of metastatic cancer cells in the cell population.

74. (Canceled)

75. (Previously presented) The method of claim 72 wherein assaying the cell population comprises incubating at least a portion of the selected cell population with a reagent capable of binding to EphA2 to allow binding of the reagent to EphA2; and detecting reagent-EphA2 binding.

76. (Previously presented) The method of claim 75 wherein the reagent is an antibody.

77. (Previously presented) The method of claim 76 wherein the antibody is produced by hybridoma D7 (ATCC number PTA 2755) or hybridoma B2D6 (ATCC number PTA 2754).

78. (Previously presented) A method for determining the disease stage in a cell population comprising cancer cells, the method comprising:

assaying at least a portion of the cell population to determine at least one of

EphA2 intracellular localization pattern; and

EphA2 phosphorylation content; and

wherein the intracellular localization pattern or phosphorylation content is indicative of the disease stage of the cancer cells.



## Amendment and Response

Page 10 of 17

Serial No.: 09/640,952

Confirmation No.: 3252

Filed: 17 August 2000

For: EPHA2 AS A DIAGNOSTIC TARGET FOR METASTATIC CANCER

79. (Previously presented) The method of claim 78 wherein assaying the cell population comprises incubating at least a portion of the cancer cell population with a reagent capable of binding to EphA2 to allow binding of the reagent to EphA2; and detecting reagent-EphA2 binding.

80. (Previously presented) The method of claim 79 wherein the reagent is an antibody.

81. (Previously presented) The method of claim 80 wherein the antibody is produced by hybridoma D7 (ATCC number PTA 2755) or hybridoma B2D6 (ATCC number PTA 2754).

82.-89. (Canceled)

90. (Previously presented) A method for detecting the presence of cancer cells in a selected cell population comprising:

assaying at least a portion of the selected cell population for at least one of

a change in EphA2 intracellular localization pattern; and

a change in EphA2 phosphorylation content

as compared to the EphA2 intracellular localization pattern and phosphorylation content in an analogous normal cell population; wherein the assaying the cell population comprises incubating at least a portion of the selected cell population with a monoclonal antibody that specifically binds EphA2 to allow antibody binding to EphA2, and wherein the change is indicative of the presence of a cancer cell in the selected cell population.

91. (Previously presented) The method of claim 90 further comprising assaying at least a portion of the selected cell population for a change in EphA2 expression level as compared to the EphA2 expression level in an analogous normal cell population, wherein a change in EphA2 expression level is indicative of the presence of nonmetastatic cancer cells in the selected cell population.

## Amendment and Response

Page 11 of 17

Serial No.: 09/640,952

Confirmation No.: 3252

Filed: 17 August 2000

For: EPHA2 AS A DIAGNOSTIC TARGET FOR METASTATIC CANCER

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92. (Previously presented) A method for detecting the presence of metastatic cells in a cell population comprising the steps of:

lysing at least a portion of the cell population;

incubating the lysed cells with an antibody produced by hybridoma D7 (ATCC number PTA 2755) or hybridoma B2D6 (ATCC number PTA 2754) to allow antibody binding to EphA2; and

detecting antibody-EphA2 binding;

wherein antibody-EphA2 binding is indicative of the presence of metastatic cells in the cell population.

93. (Previously presented) A method for detecting the presence of metastatic cells in a cell population comprising:

incubating at least a portion of the cell population with an antibody produced by hybridoma D7 (ATCC number PTA 2755) or hybridoma B2D6 (ATCC number PTA 2754) to allow antibody binding to EphA2; and

detecting antibody-EphA2 binding;

wherein antibody-EphA2 binding is indicative of the presence of metastatic cells in the cell population.

94. (Previously presented) A method for detecting the presence of cancer cells in a selected cell population comprising:

incubating at least a portion of the selected cell population with an antibody produced by hybridoma D7 (ATCC number PTA 2755) or hybridoma B2D6 (ATCC number PTA 2754) to allow binding of the antibody to EphA2; and

detecting antibody-EphA2 binding so as to detect at least one of

a change in EphA2 intracellular localization pattern; and

a change in EphA2 phosphorylation content

## Amendment and Response

Page 12 of 17

Serial No.: 09/640,952

Confirmation No.: 3252

Filed: 17 August 2000

For: EPHA2 AS A DIAGNOSTIC TARGET FOR METASTATIC CANCER

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as compared to the intracellular localization pattern and phosphorylation content in an analogous normal cell population; wherein the change is indicative of the presence of a cancer cell in the selected cell population.

95. (Previously presented) A method for determining the disease stage in a cell population comprising cancer cells, the method comprising:

incubating at least a portion of the selected cell population with an antibody produced by hybridoma D7 (ATCC number PTA 2755) or hybridoma B2D6 (ATCC number PTA 2754) to allow binding of the antibody to EphA2;

detecting antibody-EphA2 binding so as to detect at least one of

EphA2 intracellular localization; and

EphA2 phosphorylation content; and

wherein the intracellular localization pattern or phosphorylation content is indicative of the disease stage of the cancer cells.

96. (Previously presented) A method for detecting the presence of cancer cells in a selected cell population comprising:

incubating at least a portion of the selected cell population with an antibody produced by hybridoma D7 (ATCC number PTA 2755) or hybridoma B2D6 (ATCC number PTA 2754) to allow binding of the antibody to EphA2;

detecting antibody-EphA2 binding so as to detect at least one of

a change in EphA2 expression level;

a change in EphA2 intracellular localization pattern; and

a change in EphA2 phosphorylation content

as compared to the EphA2 expression level, intracellular localization pattern and phosphorylation content in an analogous normal cell population; wherein the change is indicative of the presence of a cancer cell in the selected cell population.

## Amendment and Response

Page 13 of 17

Serial No.: 09/640,952

Confirmation No.: 3252

Filed: 17 August 2000

For: EPHA2 AS A DIAGNOSTIC TARGET FOR METASTATIC CANCER

---

97. (Previously presented) The method of claim 1 wherein the cell population comprises breast cancer cells.
98. (Previously presented) The method of claim 1 wherein the cell population comprises kidney cancer cells.
99. (Previously presented) The method of claim 1 wherein the cell population comprises prostate cancer cells.
100. (Previously presented) The method of claim 1 wherein the cell population comprises lung cancer cells.
101. (Previously presented) The method of claim 1 wherein the cell population comprises colon cancer cells.